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Kinetics of the local cellular response in the gastric lymph of immune and susceptible sheep to infection with *Teladorsagia circumcincta*

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Abstract

Groups of yearling sheep were trickle infected with *Teladorsagia circumcincta* for 8 weeks, then the infection cleared with anthelmintic and both these animals and a group of parasite naïve sheep were challenged with 50 000 infective *T. circumcincta* larvae. The previously infected sheep demonstrated acquired immunity to the parasite, manifested by reduced worm burdens which were evident as early as 2 days after challenge. Cannulation of the common efferent gastric lymph duct allowed the kinetics of their local cell traffic to be monitored, and the phenotype of these lymphocytes was analysed. A blast cell response, consisting of both T and B lymphocytes, was observed in both groups of sheep, however this occurred more rapidly in the previously infected, immune animals. CD4+, CD8+ and CD25+ blast cell output peaked at day 3 in the previously infected animals, whereas CD21+ blast cell output peaked slightly later at day 5. In the control group the peak output of all phenotypes of blast cells occurred more slowly, peaking 10 days after infection.

Introduction

Teladorsagia circumcincta is an abomasal nematode parasite of sheep, which is well recognized as an important pathogen in temperate regions, both in terms of animal welfare and economic loss. Current control methods rely on the use of anthelmintic drugs; however, parasite resistance to these is widespread and increasing, and there are now reports of isolates of *T. circumcincta* which are resistant to several classes of anthelmintic (1). As sheep can acquire immunity to *T. circumcincta* (2–5), vaccination may be an alternative prospect for future control (6–9); however, the exact effector mechanisms of immunity remain to be elucidated.

Immunity to *T. circumcincta* manifests itself in several ways, including worm loss, inhibited development of larvae at the early fourth stage (EL4), stunted growth of developing worms and reduced fecundity (10,11). Possible roles for both immediate hypersensitivity responses and antibodies have been proposed. For example, IgE responses have been correlated with reduced faecal egg counts in grazing lambs (12,13), whilst IgA, the main mucosal antibody isotype, has been associated with reduced adult worm length and fecundity (10,11,14,15). In the experimental infection model employed here, the secondary IgA response observed in the gastric lymph of immune animals has been shown to occur rapidly enough to be involved in the stunting of developing worms, but too slowly to be responsible for worm loss or arrestment at the early L4 stage (11). The cellular immune response has also been demonstrated to be an important aspect of acquired immunity through adoptive transfer experiments, in which efferent gastric lymphocytes from immune animals responding to a challenge infection conferred partial protection to genetically identical naïve sheep (16). Experiments looking at the cellular profiles in the abomasal lymph node and abomasal tissue have shown changes in the quantity and proportions of both T and B cells at 3 and 5 days after infection with *T. circumcincta* (17), however the kinetics of the various cell types involved in the local cellular response has not been investigated to date.

In the present paper, we further characterize the immune response to a challenge infection of *Teladorsagia* by monitoring the phenotype and kinetics of the cells in the efferent gastric lymph of sheep which were immune or susceptible to infection, and by determining whether immune sheep reject the challenge infection within 48 h.

Materials and Methods

Sheep

Three experiments were conducted using a total of 48 yearling sheep aged 10–12 months. All had been reared indoors under conditions designed to exclude accidental infection with nematode parasites.

Infective larvae

Infective L3 were from an anthelmintic susceptible *T. circumcincta* isolate which had been passaged through sheep at Moredun Research Institute for a number of years. Larvae were stored for up to 1 month at 4°C prior to administration. All infective larvae used within each experiment were derived from the same batch.

Surgical procedures, sample collection and post-mortem procedures

The common gastric lymph duct, which contains efferent lymph draining all four stomachs, was cannulated as detailed elsewhere (18). The sheep were fitted with an indwelling venous catheter placed in the jugular vein or the posterior vena cava. Collection, sampling and re-infusion of lymph, and post-mortem procedures were carried out as previously reported (11).

Worm counting and staging

Sub-samples (5%) of abomasal washings and mucosal digests were stained by the addition of a concentrated iodine solution, washed over a 38 µm sieve and searched under a stereomicroscope. The number of male, female and sexually undifferentiated EL4 was recorded.

Statistical methods

Arithmetic mean with standard errors are shown throughout. Parasite counts and percentage EL4 were compared by Student's *t*-test. Cell numbers were compared using Student's *t*-test, and, after log transformation, by repeated measure models (Genstat, VSN International Ltd, Hemel Hempstead, UK). $P < 0.05$ is regarded as the significant level of probability throughout.

Design of experiments

Three trials designated Experiments 2, 3 and 4 were conducted (Table 1), so numbered because they were part of a larger series of trials sharing the same general design (11).

All three experiments contained a group of previously infected sheep which had received a trickle immunizing infection of 2000 infective *Teladorsagia* larvae three times per week for 2 months. Experiments 2 and 4 also contained control sheep which did not receive the trickle infection. All sheep were challenged with a single dose of 50 000 *Teladorsagia* larvae 7 days after receiving fenbendazole to remove any remnants of the trickle infection from the previously infected animals. In experiments 2 and 3 the gastric lymph ducts of 10 sheep in each group were cannulated in the interval between anthelmintic treatment and challenge.

Experiment 2, which contained 30 sheep, has been described in detail before [Experiment 2 in (11)]. It compared the number and state of development of worms recovered from previously infected or control sheep killed 5, 10 or 21 days following challenge (Table 1) in relation to their lymph IgA responses. Here, we describe changes in the lymph cell traffic of three previously infected and four control sheep from that trial for 10 and 21 days after challenge, respectively. Previously infected sheep were killed 10 days after challenge as it is known from prior experiments using this model that the major manifestations of immunity to the parasite (worm loss and arrested development), as well as the cellular and humoral responses occur within 10 days (3,11).

Experiment 3 consisted of six previously infected sheep killed 10 days after challenge. Gastric lymph was successfully obtained from four of these and their cell traffic data was pooled with that of the three previously infected sheep in Experiment 2.

Experiment 4 contained six previously infected and six control sheep. Both groups were killed 2 days after challenge to determine whether worm loss had occurred by this time point. Lymph was not collected during this experiment.

Immunofluorescent surface staining of lymphocytes

Lymph was diluted 1 : 500 in Coulter[®] Isoton[®] II Diluent (Beckman Coulter Inc., Fullerton, CA, USA) using a Coulter Diluter (Coulter Electronics Ltd, Luton, UK) and total (>3 µm diameter) and large (>9 µm diameter) lymphocyte numbers counted using a Z1 Coulter[®] Particle Counter (Beckman Coulter) Counter. 1×10^7 cells were centrifuged for 5 min at 1500 rpm, and the pellet washed by resuspending in 5 mL FACS buffer [PBS, 5% (v/v) foetal calf serum, 0.02% (w/v) NaN₃] and centrifuging again at 1500 rpm for 5 min.

After a further wash in 5 mL FACS buffer the final pellet was resuspended in 1 mL FACS buffer. Live/dead discrimination using phase contrast was performed on early efferent lymph samples before staining and showed very little cell death, and examination of nigrosin stained lymphocytes also revealed negligible cell death. Fifty microlitres washed lymphocytes, at 1×10^7 cells/mL, were added to round-bottomed 96-well plates (Bibby Sterilin Ltd, Staffs, UK) and spun for 1 min at 2000 rpm. The supernatant was discarded, and after vortexing briefly to resuspend the pellet 50 μ L per well of primary antibody diluted in FACS buffer was added. Monoclonal antibodies that recognize border disease virus as isotype controls [clones VPM21 (isotype IgG1, 1/500 dilution) and VPM22 isotype IgG2a, 1/500 (19)], ovine CD2 [clone 36F, isotype IgG2a, 1/1000 (20)], CD4 [clone 17D, IgG1, 1/1000 (21)], CD8 [clone 7C2, IgG2a, 1/1000 (22)], $\gamma\delta$ -T cell receptor [clone 86D, IgG1, 1/1000 (23)], CD25 [clone ILA111, IgG2a, 1/2000 (24)], major histocompatibility complex class II [MHC-II; clone VPM46, IgG2a, 1/1000 (25)], CD21 [clone CC21, IgG1, 1/10 (26)] and IgA (MCA628; Serotec, Oxford, UK, IgG1, 1/1000) were used. Plates were incubated for 20 min at 4°C, centrifuged for 1 min at 2000 rpm, the supernatant discarded, then after vortexing briefly wells were washed twice with 200 μ L FACS buffer. After the second wash, 50 μ L goat anti-mouse Ig phycoerythrin conjugate secondary antibody (Invitrogen Corporation, San Diego, CA, USA) diluted 1/400 in FACS buffer were added to each well. Plates were incubated in the dark for 15 min at room temperature, then 150 μ L FACS buffer was added to each well before centrifuging for 1 min at 2000 rpm. Wells were then washed in 200 μ L FACS buffer then 200 μ L PBS before fixing in 1% (w/v) paraformaldehyde in PBS and analysed within 24 h.

Flow cytometry for visualization of stained cells

Fluorescent data were acquired on a FACScan flow cytometer equipped with a 488 nm argon-ion laser and analysed using CellQuest software (Becton Dickinson, Franklin Lakes, NJ, USA). A minimum of 10 000 cells were acquired for each sample. Lymphocytes were initially gated according to forward scatter and side scatter to eliminate debris and dead cells, then percentage positive for each marker calculated. Large cells were defined by a visually determined cut off according to forward scatter. Example FACS plots from one previously infected sheep at days 1 and 3 post-challenge are shown in Figure 1. An initial Fsc vs. Ssc plot was gated for small lymphocytes, R1; blasting lymphocytes (lymphoblasts), R2; and total lymphocytes, R3 (R3 = R1 + R2) (Figure 1a and b). Negative control samples with no

primary antibody (Figure 1c and d), and isotype controls (Figure 1e and f) were then plotted as Fsc vs. fluorescence, gated with R3. Quadrants were set with the Fsc cut-off for large cells as established during initial gating. Cells in the upper quadrants represent those positive for the staining antibody, and those in the upper right positive blast cells. The percentage of total cells positive for the isotype control antibodies was observed to be below 0.1% for almost all samples. Cells stained with anti-CD4, gated with R3, are shown in Figure 1g and h.

Results

In Experiments 2 and 4 significantly ($P < 0.01$) fewer worms were recovered from the previously infected sheep compared to their respective controls at each time point (Figure 2) and the number recovered from the previously infected sheep in Experiment 3 was consistent with this data. This indicated that the previously infected sheep rejected the challenge larvae within 48 h.

Lymph flow

Lymph was collected until day 21 from 4 control sheep, and, except for 1 animal which stopped flowing on day 7, until day 9 or 10 from 7 previously infected sheep. Data from the sheep which stopped flowing at day 7 has been included in the group mean calculations for the relevant time points.

The overall mean lymph flow rate was 14.8 ± 0.8 mL/h. No significant changes with time or between groups were observed.

Lymph cell output

Total cells

In the control sheep the mean rate of total cell output increased from $1.55 \pm 0.31 \times 10^8$ cells/h on day 0 to $2.63 \pm 0.31 \times 10^8$ cells/h by day 3, however this was not a significant increase ($P = 0.08$) (Figure 3a). The mean rate of total cell output then remained elevated until day 10, thereafter falling to between 1.0 and 1.5×10^8 /h, similar to prechallenge values.

In previously infected sheep mean total cell output was $2.96 \pm 0.62 \times 10^8$ cells/h on day 3 compared to $1.86 \pm 0.63 \times 10^8$ cells/h on day 0, a significant increase ($P < 0.05$). No

significant difference was found between the groups. Total cell output appeared to decrease slightly in both groups towards the end of the experiment.

Percentage blast cells

Prior to the challenge infection the proportion of lymphoblasts was similar in both groups with a mean of about 3% (Figure 3b). After challenge this value increased steadily in both groups, increasing to approximately 12% on day 8 in the previously infected, and peaking at approximately 14% on day 17 in the controls. No significant differences were observed between the groups except on day 1 when the percentage was significantly higher ($P < 0.05$) in the previously infected sheep.

Total lymphoblasts

In the previously infected sheep the rate of lymphoblast output increased significantly ($P = 0.05$) from $0.95 \pm 0.36 \times 10^7$ cells/h on day 1 to peak at $2.10 \pm 0.46 \times 10^7$ cells/h on day 3, then decreased almost to prechallenge levels by day 8 (Figure 3c). In the control sheep lymphoblast output rose more slowly after challenge, from $0.31 \pm 0.07 \times 10^7$ cells/h on day 1 to a peak of $2.91 \pm 0.47 \times 10^7$ cells/h ($P = 0.01$) on day 10, and then decreased throughout the remainder of the experiment (Figure 3c). As the most significant changes occurred within the blast cell population, and these are most likely to be the cells responding to the parasite infection, the cell-surface marker staining results are focused on lymphoblasts.

Proportion of T and B lymphocytes

In the previously infected group, the mean proportion of total lymphocytes expressing the cell surface markers CD4, CD8, γ/δ T-cell receptor and CD21 at 1 day post-challenge was 50%, 21%, 10% and 15% respectively, and in the control group was 54%, 17%, 9% and 30% (Table 2). Large between animal variation was observed, however the proportions of cell types in the efferent lymph did not differ between the two groups. Other than a slight decrease in CD4⁺ cells towards the end of the experiment in both groups, no changes over time in the proportion of cells expressing these markers was observed.

T cells

The response of lymphoblasts expressing the pan T-cell marker, CD2, was significantly different ($P = 0.032$) between the two groups (Figure 4a). CD2⁺ blasting cells in the

previously infected group rose from $0.43 \pm 0.15 \times 10^7$ cells/h on day 1 to peak on day 3 at $1.38 \pm 0.33 \times 10^7$ cells/h, while in the control group they initially increased more slowly, from $0.18 \pm 0.03 \times 10^7$ cells/h on day 1, peaking on day 10 at $2.36 \pm 0.60 \times 10^7$ cells/h. In each group the peak CD2⁺ lymphoblast output was significantly higher than at 1 day post-challenge ($P < 0.05$).

Similar between group differences were observed for CD4⁺ ($P = 0.049$, Figure 4b) and CD25⁺ ($P = 0.017$, Figure 4c) lymphoblast outputs which peaked in the previously infected group on day 3 at 1.02 ± 0.27 , and $0.97 \pm 0.24 \times 10^7$ cells/h respectively. In contrast, CD4⁺ and CD25⁺ lymphoblasts in the control group did not peak until day 10 with outputs of 1.22 ± 0.17 and $1.77 \pm 0.26 \times 10^7$ cells/h respectively. In each group of sheep peak outputs of both cell types were significantly ($P < 0.05$) greater than the corresponding day 1 values. In addition, the mean peak CD25⁺ output from the naïve group was significantly higher than from previously infected group ($P = 0.05$).

The CD8⁺ lymphoblast response was also significantly different between the two groups ($P = 0.017$), with positive blasting cells in the previously infected group again peaking 3 days post-challenge at $0.26 \pm 0.07 \times 10^7$ cells/h (Figure 4d). However, this was not significantly different from the day 1 output of $0.18 \pm 0.07 \times 10^7$ cells/h ($P = 0.08$), possibly due to wide individual variation. In the control group, a trend similar to that seen with CD4⁺ and CD25⁺ blasts was observed, with the CD8⁺ lymphoblast output peaking at day 10 at $0.97 \pm 0.39 \times 10^7$ cells/h. However, statistical analysis again showed this to be not significantly more than day 1 levels ($P = 0.1$), and also not significantly more than the peak output of the previously infected group ($P = 0.17$), possibly due to the high degree of individual variation observed and the relatively small group size.

The pattern of gamma-delta T-cell receptor positive lymphoblast response was significantly different between the two groups over the first 8 days of infection ($P = 0.001$, Figure 4e), with increased output in the previously infected group at days 1 and 3 compared to the control group. Analysis of the control group over the whole experiment showed that levels of γ/δ TCR⁺ blast cells did not change significantly with time.

B cells

Absolute numbers of blasting cells expressing B-cell markers are shown in Figure 5, with lymphoblasts expressing CD21 shown in Figure 5(a). In the previously infected group, CD21⁺ blast cell output rose slightly from $0.28 \pm 0.14 \times 10^7$ cells/h on day 1 to

$0.49 \pm 0.19 \times 10^7$ cells/h on day 5, then dropped to $0.18 \pm 0.09 \times 10^7$ cells/h on day 8, but these changes were not significant. In the control group, CD21⁺ blast cell output rose significantly ($P = 0.01$) from $0.13 \pm 0.04 \times 10^7$ cells/h on day 1 to $1.04 \pm 0.17 \times 10^7$ cells/h on day 10, before decreasing back to prechallenge levels by day 19.

In the previously infected group, IgA⁺ blast cell output peaked at day 5 at $0.41 \pm 0.14 \times 10^7$ cells/h, significantly higher ($P = 0.04$) than day 1 ($0.09 \pm 0.03 \times 10^7$ cells/h), then dropped back to $0.12 \pm 0.06 \times 10^7$ cells/h by day 8 (Figure 5b). In the control group the IgA⁺ blast cell response appeared to rise slowly over time, from $0.08 \pm 0.04 \times 10^7$ cells/h on day 1 to $0.29 \pm 0.07 \times 10^7$ cells/h on day 22, the only point at which the output was significantly higher than day 1 ($P = 0.04$).

Figure 5(c and d) show the kinetics of the IgA⁺ blast cell response in relation to total lymph IgA concentrations which have been reported previously (11). In control sheep mean total IgA concentrations did not change significantly over the course of the experiment reflecting the general lack of an IgA⁺ blast cell response. In contrast a clear anamnestic total IgA response was observed in the previously infected sheep, which tracked the secondary IgA⁺ blast cell response.

MHC II positive cells

The response of lymphoblasts expressing MHC II on the cell surface is shown in Figure 5(e). Prechallenge this was in the range $0.3\text{--}0.6 \times 10^7$ cells/h, and was not different between the two groups. After challenge MHC 11⁺ lymphoblast output increased in both groups, peaking at day 3 in the previously infected sheep at $1.26 \pm 0.29 \times 10^7$ cells/h, and at day 10 in the naïve animals at $2.06 \pm 0.43 \times 10^7$ cells/h before subsiding again.

Discussion

The parasitology data from the three trials described in the present paper confirmed that, with this particular infection model, the previously infected sheep rejected most of the challenge dose which established in the control animals (11) and showed that this phenomenon had occurred within 2 days of infection. Worm loss, through exclusion or expulsion of incoming larvae from the gastric pit, is the most important manifestation of immunity to *T. circumcincta*, and prior experiments suggested that it was the result of an active immune response rather than a passive state (10). In each group, more worms were recovered at 10 days post-infection than on day 5, which probably reflects the relative inefficiency of recovering worms from the gastric mucosa, as experienced previously (16).

In this paper, we followed the kinetics of the local cellular response in the efferent gastric lymph to infection with *T. circumcincta*. An increase in total lymphocyte output was observed in both groups after infection, corresponding to the 'recruitment phase' of the local inflammatory response (27). An increase in the percentage of large, blast cells in the gastric lymph, that is, cells responding to the parasitic infection, was also observed in both groups, however this occurred more rapidly in previously infected than in naïve animals. When this data was combined with the total cell output to calculate absolute blast cell traffic, the previously infected animals showed a rapid increase in large cell output, which peaked 3 days after challenge. A significant increase in blast cell output was also observed in the animals undergoing primary infection, however it occurred much more slowly, not peaking until day 10. This agrees with previous findings where the local cellular response of previously infected sheep was observed to peak sooner than that of sheep undergoing a primary infection (2,3).

As expected, the cells in the efferent gastric lymph were almost exclusively lymphocytes; no cells positive for monocyte or macrophage specific markers were observed (data not shown). The proportion of T and B cells in efferent gastric lymph was comparable to that reported in lymph draining other lymph nodes (28,29), including some draining other mucosal surfaces (27,28,30,31). A slight drop in the percentage of CD4⁺ cells was observed in both groups towards the end of the experiment (data not shown), however other than this the cellular response manifested as an increase in total output of cells, and in particular blast cells, rather than as a change in the proportion of individual cell types.

Phenotypic analysis of the cells involved in the immune response, peaking at day 3 in previously infected sheep and day 10 in naïve sheep, showed that they were predominantly T cells, and within this subset mainly CD4⁺. Depletion experiments have shown CD4⁺ T cells

to be essential for immunity of sheep to *Haemonchus contortus* (32–34), and while similar experiments have not, to our knowledge, been carried out with *T. circumcincta*, our results suggest that CD4⁺ cells will also play an important role in immunity to this parasite.

CD4⁺ cell outputs were greater than CD8⁺ during the blast cell response of the previously infected sheep but peak CD8⁺ blast cell output was slightly higher in the primary infection, suggesting a predominant role for CD4⁺ cells in the memory response. CD25⁺ lymphoblast numbers corresponded to T lymphoblast numbers, which could reflect either T cells expressing CD25 (IL-2R) upon activation, or regulatory T cells, however labelling of multiple cell surface markers would be required to determine which.

The B-cell response occurred slightly later than the T-cell response, peaking 5 days after challenge in the previously infected animals. The peak IgA⁺ blast cell response almost matched the total B-cell blasts in this group, most likely because isotype switching to IgA had occurred during the trickle infection period. The same sheep also demonstrated a secondary lymph IgA response (11), which tracked these IgA⁺ blast cells approximately 2 days later. By 8 days post-challenge, IgA⁺ blast cell output had returned to prechallenge levels even although the amount of IgA in the lymph remained elevated. Presumably the source of the lymph IgA was plasma cells in the mucosa derived to at least some extent from the lymph IgA⁺ blast cells which had re-circulated to the gastric mucosa. CD21⁺ blast cells also subsided to prechallenge levels by day 8. By analogy with human plasma cells, which rarely express CD21 (CR2) (29,35), this change in phenotype was probably due to the loss of CD21 from terminally differentiated antibody secreting cells although this has not yet been confirmed for the sheep.

In the naïve group, the B-cell blast response was slower, not peaking until day 10, and a corresponding peak in IgA⁺ blast cells was not observed, as these animals were undergoing a primary immune response.

In this paper, we have characterized the phenotype and extent of the cell traffic response in the gastric lymph of immune or susceptible sheep after infection with *T. circumcincta*. These responses do not seem to have been monitored in this way before in lymph draining a mucosal surface. Pernthaner *et al.* (30) monitored cells in afferent and efferent intestinal lymph from sheep infected with *T. colubriformis* but did not document any changes other than a decrease in the proportion of CD4⁺ cells from 1 week after infection. Yen *et al.* (31) collected efferent lymph draining the nasopharynx of sheep, and reported an increase in lymphocyte proliferation *ex vivo* following intranasal vaccination, but did not report any change in cell traffic.

In the present study, a memory response was observed in the immune animals consisting initially of blasting CD4⁺ cells, closely followed by IgA⁺ B cells and a corresponding increase in parasite specific lymph IgA. However, the worm loss preceded all these responses, indicating that none was directly responsible and that the immune effector mechanism was probably operating in the abomasal mucosa. The function of the lymph cell response may be to disseminate primed or memory cells to other mucosal surfaces including the abomasum, a contention supported by earlier adoptive transfer experiments (16), in which cells from immune sheep undergoing a response to infection conferred partial immunity to genetically identical, naïve sheep.

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Figures and Tables Legend

Fig.1

Example FACS plots from one previously infected sheep. a; c; e and g, 1 day post-challenge. b; d; f and h, 3 days post-challenge. a and b, Fsc vs. Ssc, no gate. R1 = small lymphocytes, R2 = lymphoblasts, R3 = total lymphocytes (R1 + R2). c and d, Fsc vs. fluorescence, cells only (negative control) sample. Gate = R3. e and f, Fsc vs. fluorescence, isotype control sample. Gate = R3. g and h, Fsc vs. fluorescence, CD4 stained sample. Gate = R3.

Fig.2

Worm burdens. Open symbols = controls. Closed symbols = previously infected. Squares = experiment 2. Circle = experiment 3. Triangles = experiment 4.

Fig.3

Cell output. Closed squares = controls. Open triangles = previously infected. (a) Total cell output per hour. (b) Percentage lymphoblasts. (c) Total lymphoblast output per hour.

Fig.4

T-cell lymphoblast output per hour. Squares = controls. Triangles = previously infected. (a) $CD2^+$. (b) $CD4^+$. (c) $CD8^+$. (d) $CD25^+$. (e) $c/d\ TCR^+$.

Fig.5

B-cell lymphoblast output per hour. Squares = controls. Triangles = previously infected. Solid lines = cells. Asterisks and broken lines = antibody. (a) $CD21^+$. (b) IgA^+ . (c) Control IgA^+ blast cells and total IgA. (d) Previously infected IgA^+ blast cells and total IgA. (e) $MHC\ 11^+$.

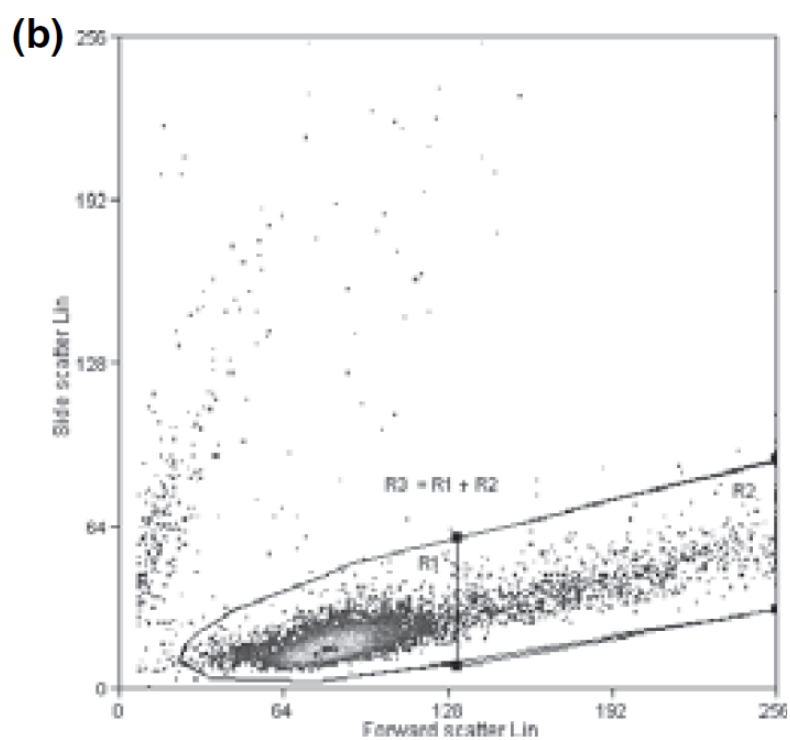
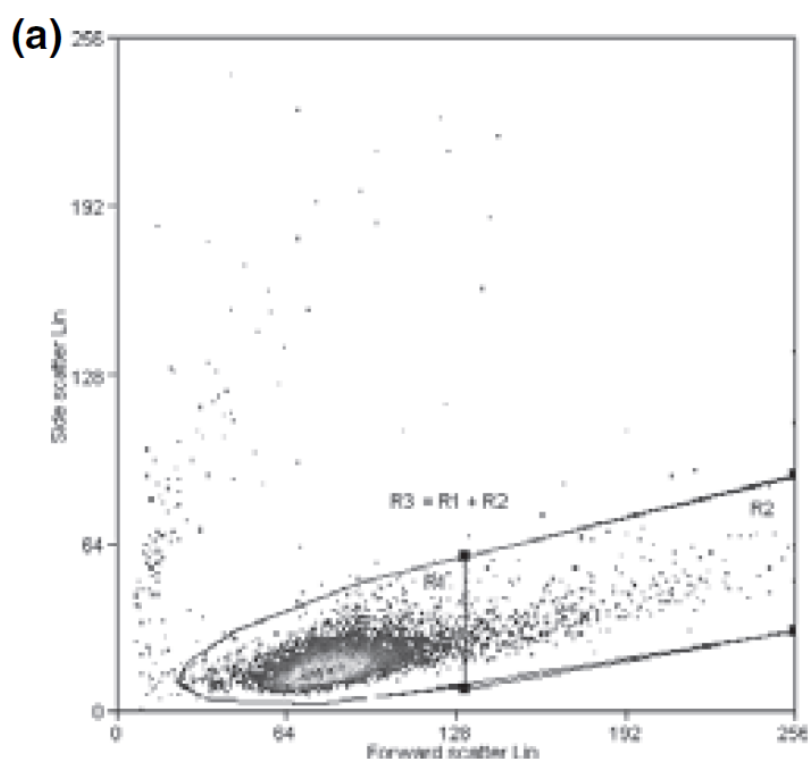
Tab.1

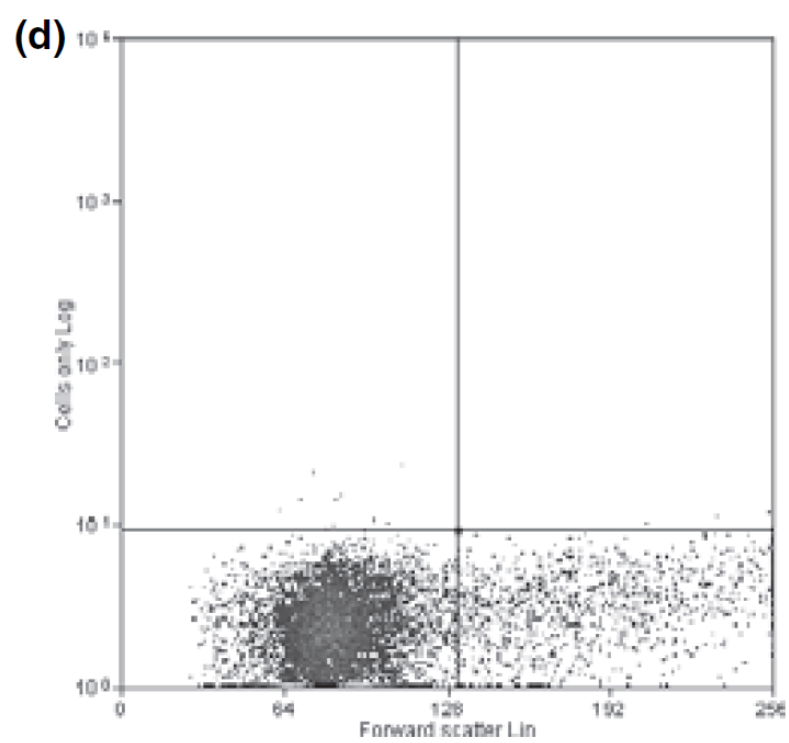
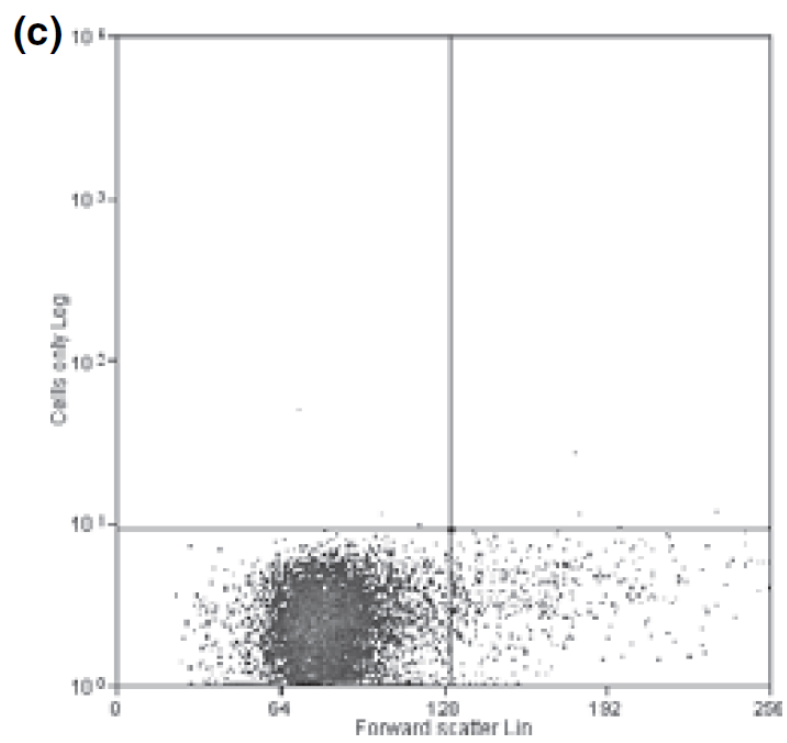
Design of experiments. ^a2000 *T. circumcincta* L3 three times per week for 8 weeks; ^b50 000 *T. circumcincta* L3. SxD = Suffolk tup, Dorset ewe; LxB = Leicester tup, Scottish Blackface ewe.

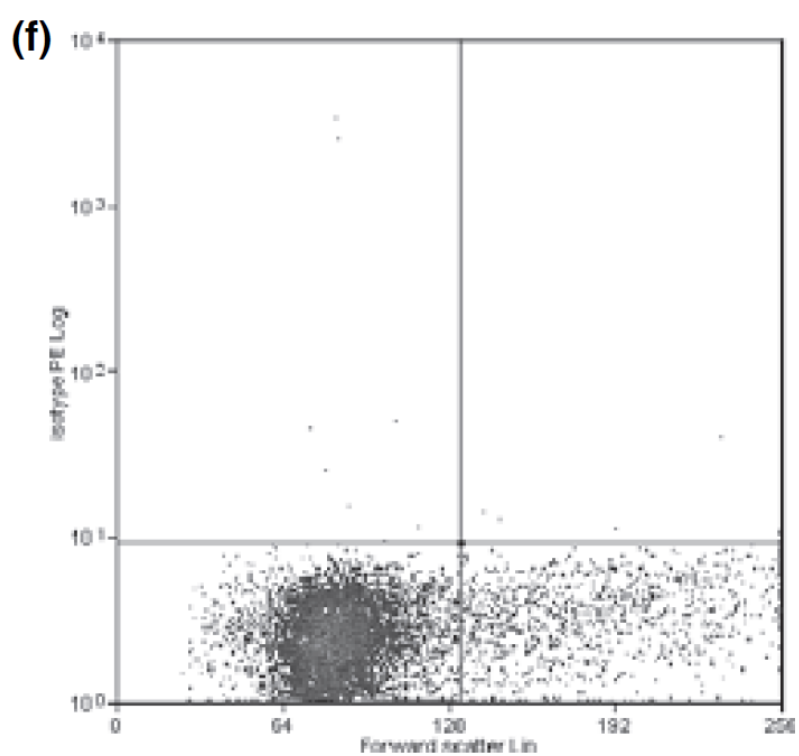
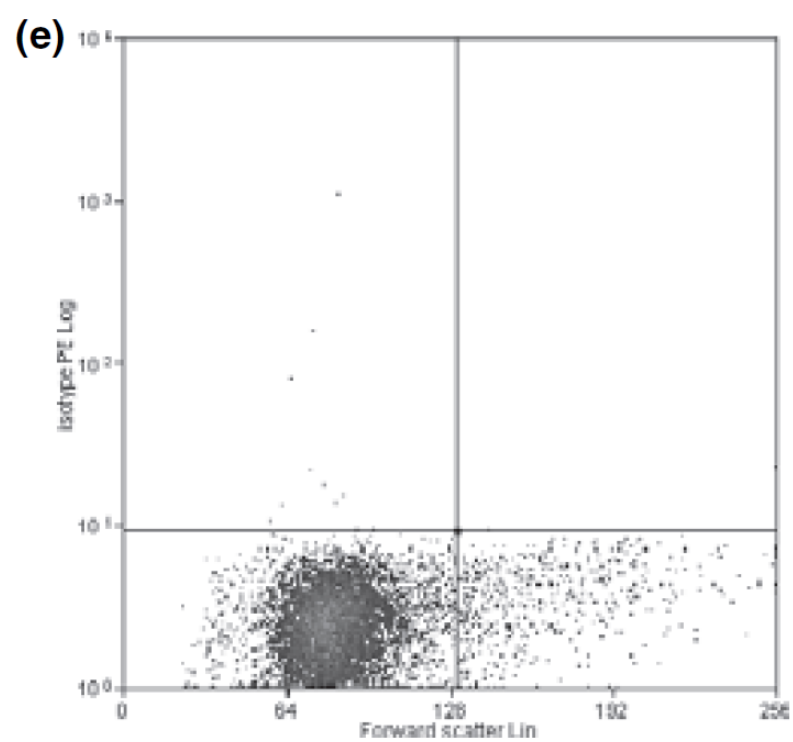
Tab.2

Distribution of lymphocyte subsets at one day postchallenge in the efferent gastric lymph of naive and previously infected sheep.

Fig.1







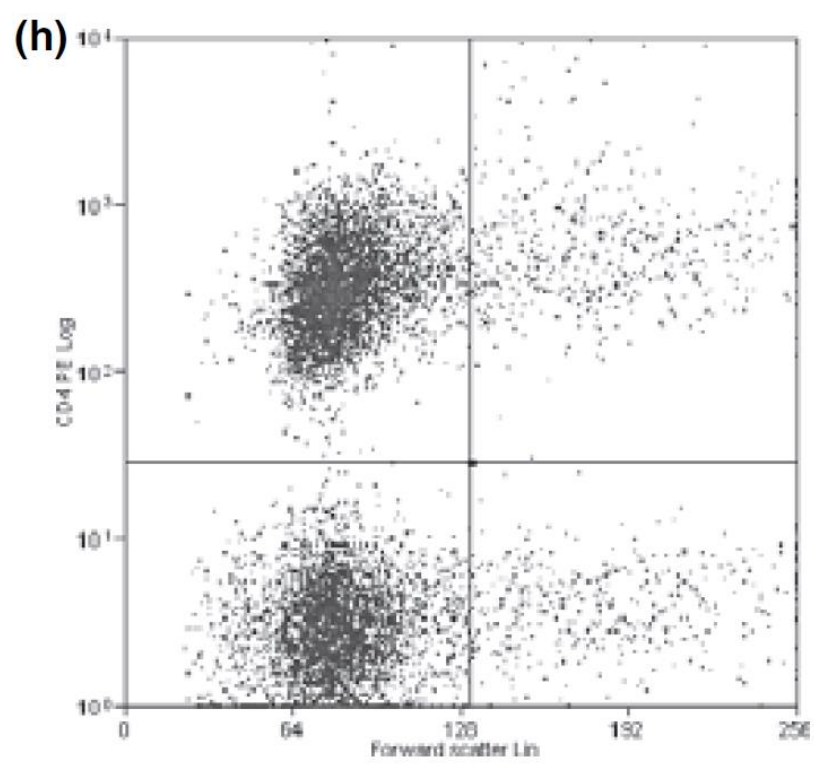
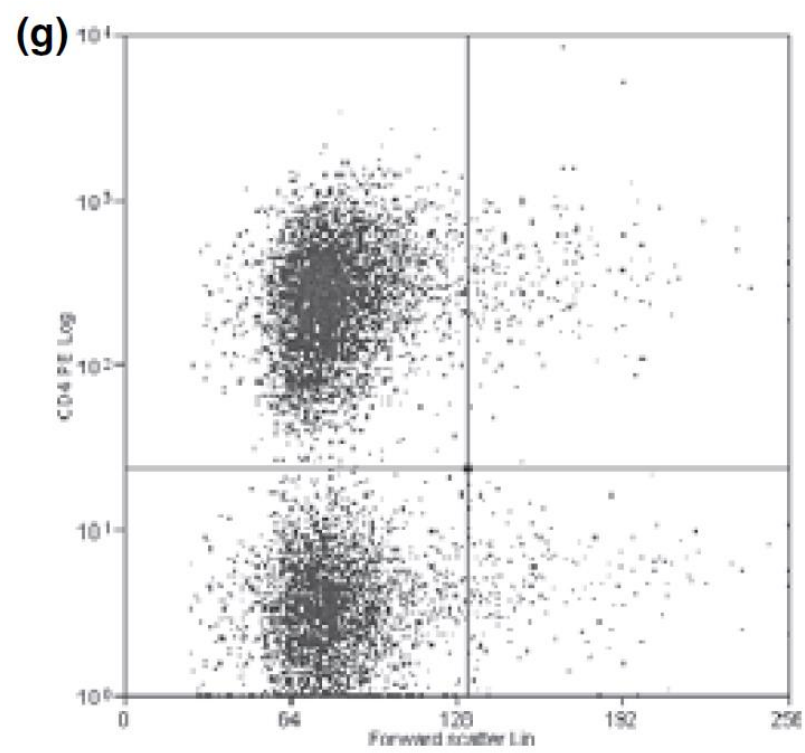


Fig.2

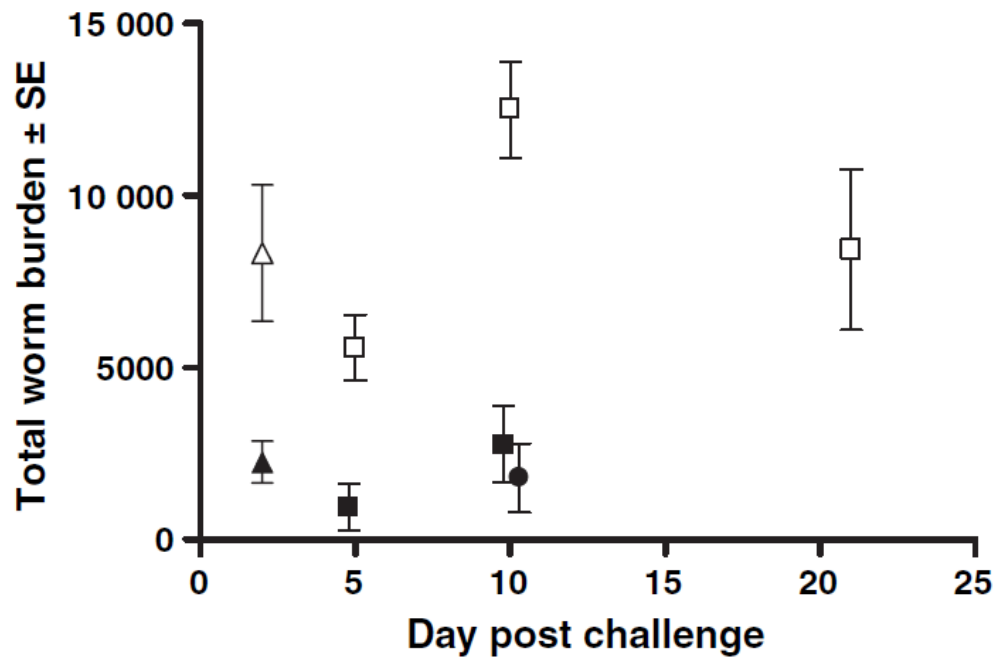
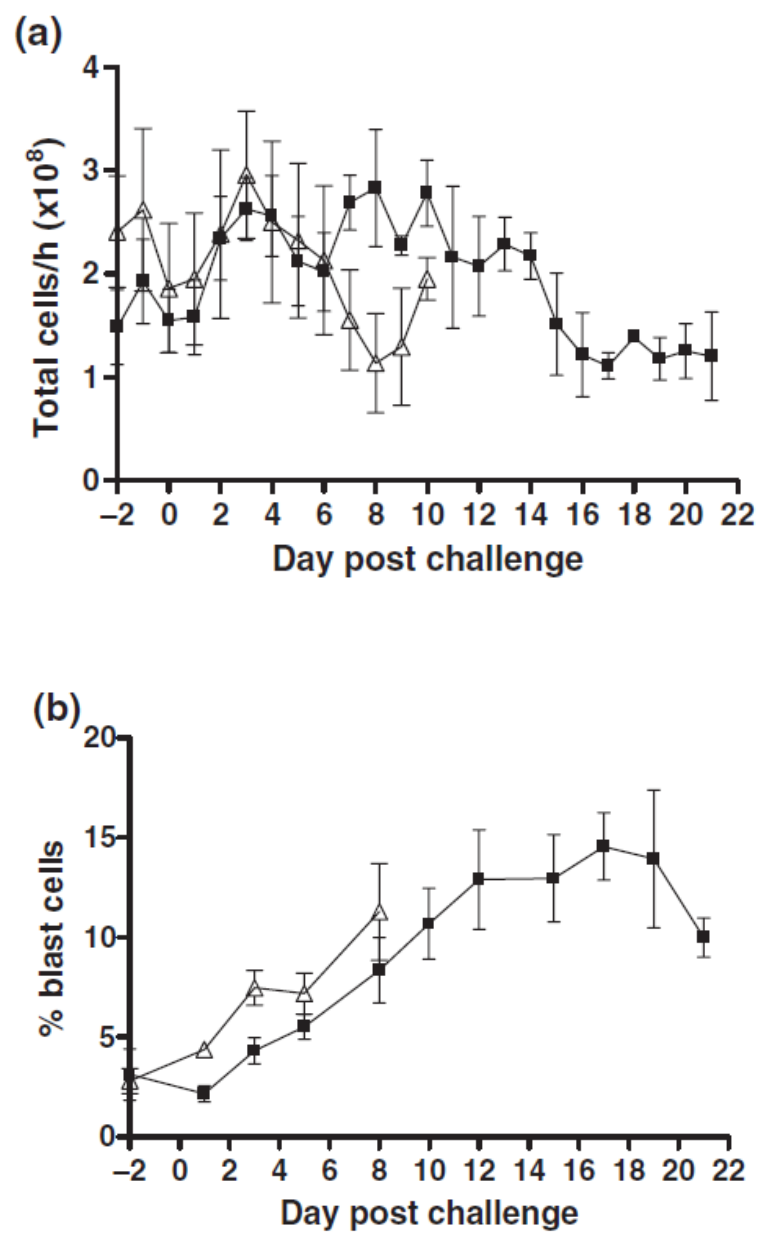


Fig.3



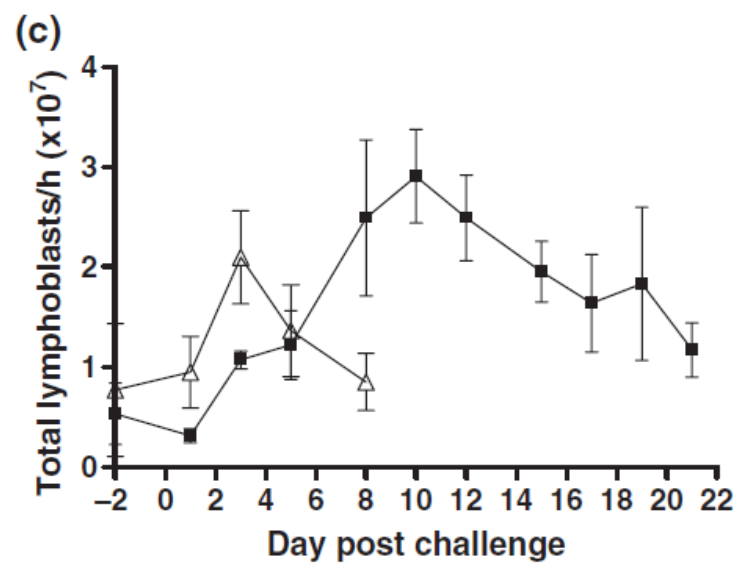
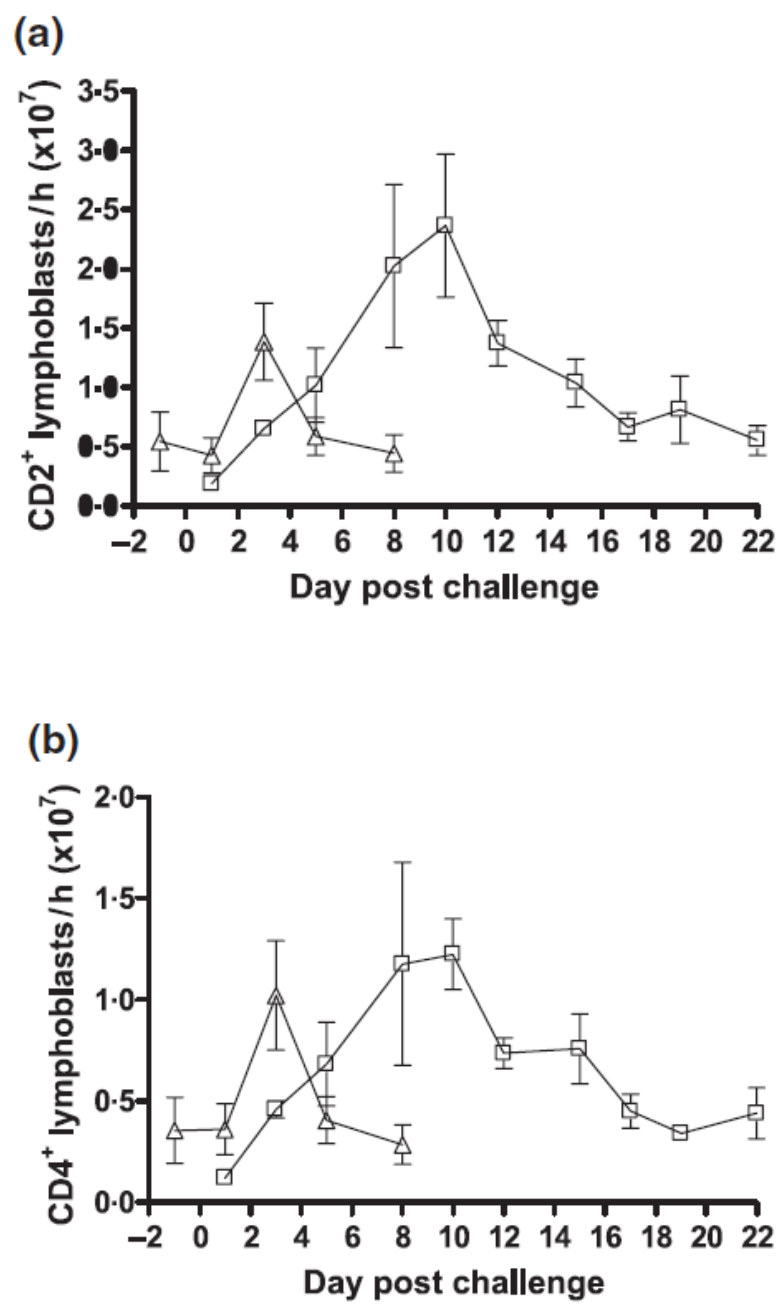
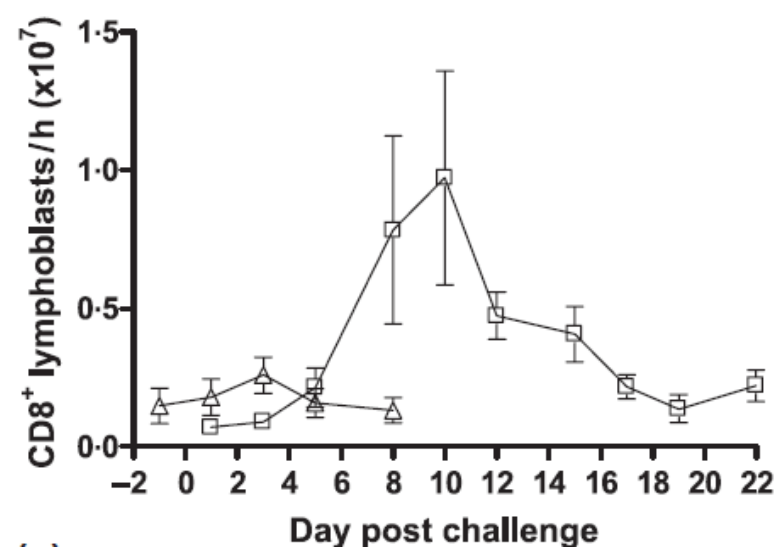


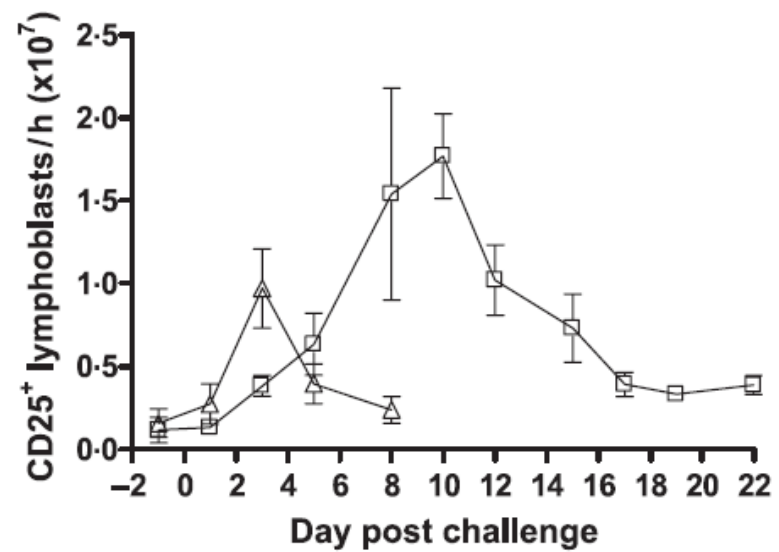
Fig.4



(c)



(d)



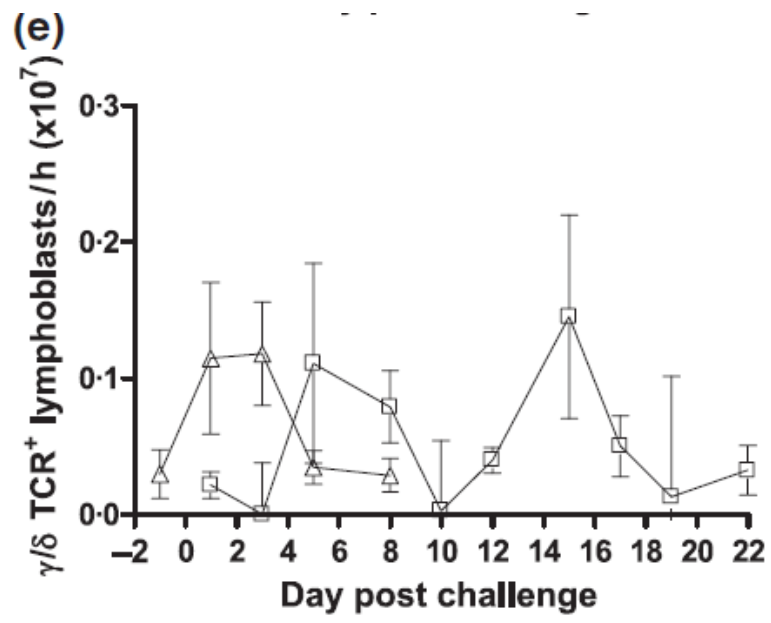
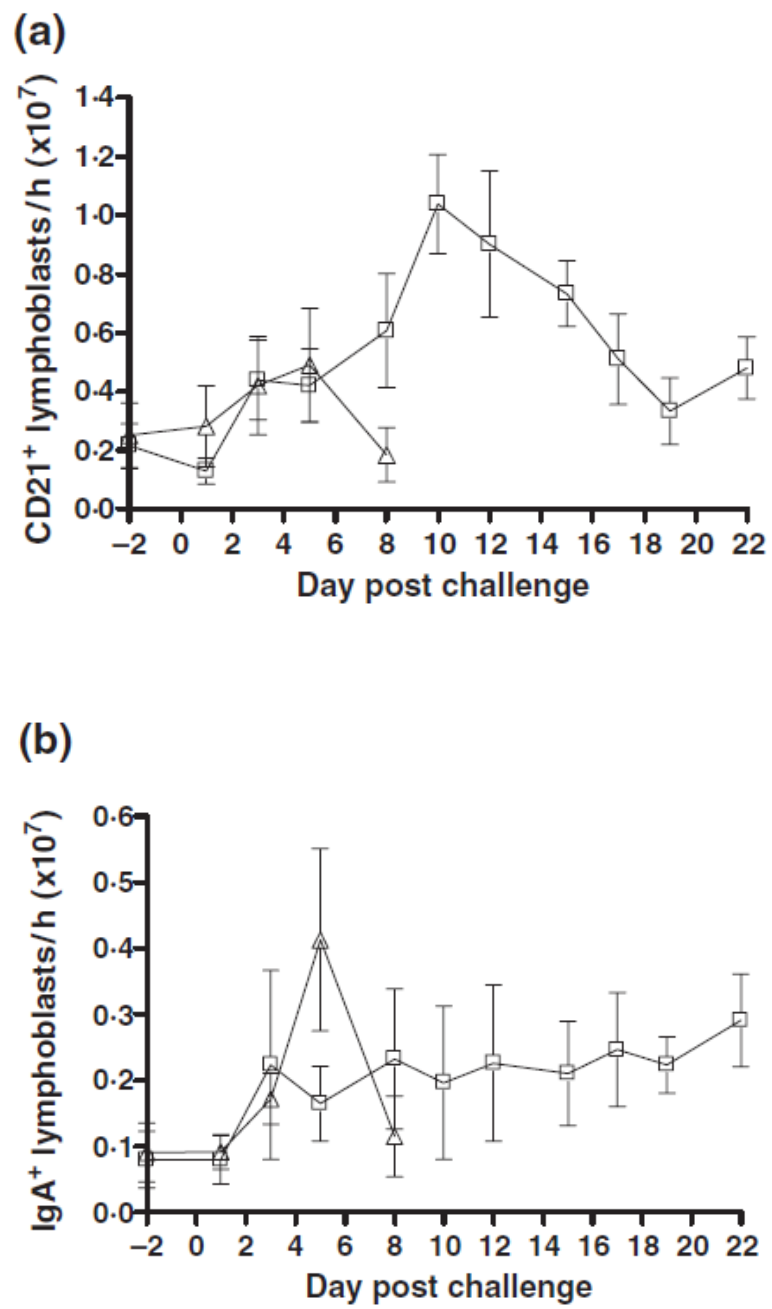
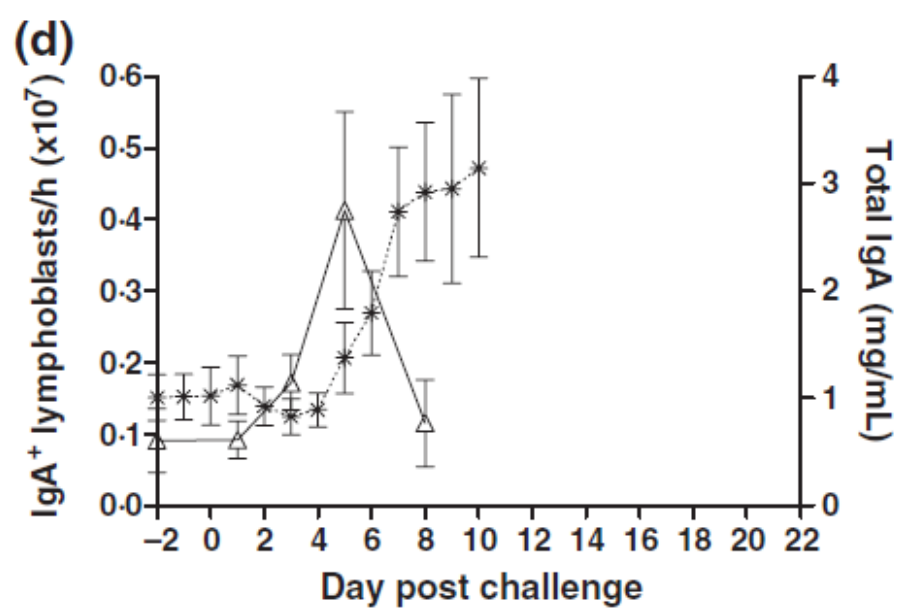
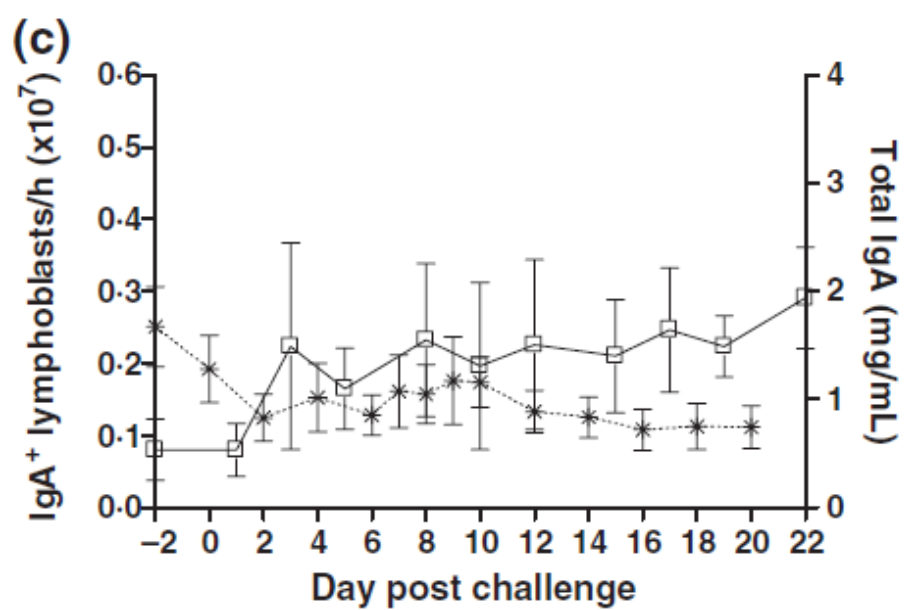
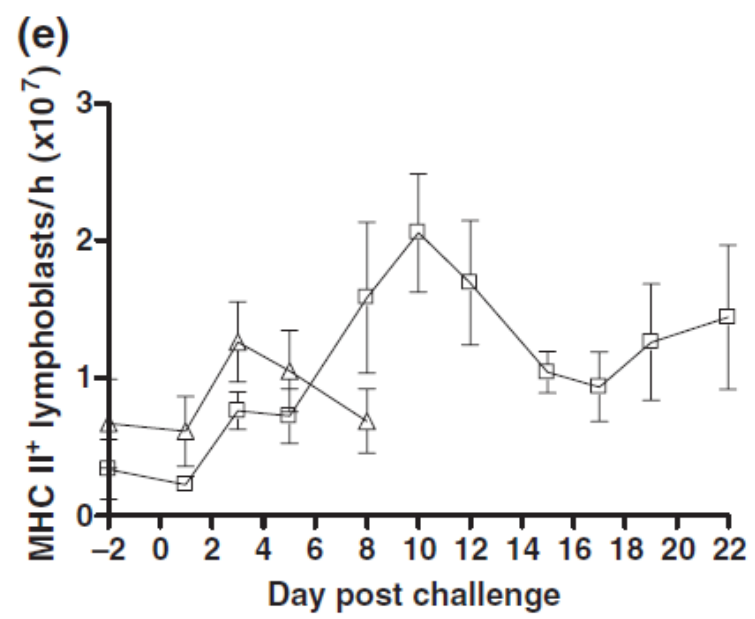


Fig.5







Tab.1

Experiment	Breed	Group	Trickle infection ^a	Treatments (relative to day of challenge)				Main purpose							
				Fenben- dazole (5 mg/kg)	Cannulate gastric lymph duct	Challenge infection ^b	Number killed								
							- 62 to - 9		- 7	- 6 to - 2	0				
												2	5	10	21
1. ^a 2000 <i>T. circumcincta</i> L3 three times per week for 8 weeks; ^b 50 000 <i>T. circumcincta</i> L3. SxD = Suffolk tup, Dorset ewe; LxB = Leicester tup, Scottish Blackface ewe.															
2	SxD	Previously infected	+		+		-	6	6	-	To provide lymph cell traffic data from immune and naïve sheep post- challenge				
		Control	-		+		-	6	6	6					
3	SxD	Previously infected	+		+		-	-	6	-	Does worm loss occur by day 2 post- challenge?				
				+		+									
4	LxB	Previously infected	+		-		6	-	-	-					
		Control	-		-		6	-	-	-					

Tab.2

Cell type	Naïve Mean % (range)	Previously infected Mean % (range)
T cells (CD2 ⁺)	71 (59–77)	71 (60–77)
CD4 ⁺	54 (48–62)	50 (41–60)
CD8 ⁺	17 (11–23)	21 (15–29)
$\gamma\delta$	9 (7–13)	10 (6–16)
B cells (CD21 ⁺)	30 (7–50)	15 (10–21)